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Improved assessment of pyrogenic carbon quantity and quality in environmental samples by high-performance liquid chromatography

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Abstract

The analysis of pyrogenic carbon (PyC) in environmental samples is of great interest, e.g. for carbon cycle assessment, (bio-)char characterization and palaeoenvironmental or archaeological reconstruction. Here, an HPLC method (HPLC) is presented that reproducibly quantifies Benzene Polycarboxylic Acids (BPCA) as molecular markers for PyC in various kinds of environmental samples. It operates at low pH without requiring an organic modifier and was thoroughly tested with PyC reference materials and a peatland core that served as a feasibility and plausibility check. Compared to the established gas chromatography (GC) method, the HPLC method results in higher BPCA quantification reproducibility by showing a significantly smaller coefficient of variation (HPLC: 5 %, GC: 16 – 23 %). It works well with small sample amounts, as for instance from sediment cores and aerosol collectors, and requires less sample preparation work than the GC method. Moreover, the here presented HPLC method facilitates ^{13}C and ^{14}C analyses on PyC from environmental samples.

Keywords

Pyrogenic organic matter, Black carbon, sediment, soil, char, BPCA

1. Introduction

Pyrogenic carbon (PyC) is the solid residue of incomplete biomass combustion and can persist in the environment for a long time [1-2]. It is, therefore, ubiquitously found in different environmental matrices, e.g. in soil, sediment, water or as an aerosol [3-5]. Its accurate quantification is of great interest because its slow turnover has implications for the global carbon budget [6]. In addition, PyC affects the atmospheric radiative budget [5] and is a constituent of many anthropogenic nanoparticles [7]. Many different methods have been developed for PyC detection and quantification [6,8-9] because PyC is not a defined chemical structure but rather a continuum of thermally altered biomass [6,10]. The benzene polycarboxylic acids (BPCA) analysis [4,11-13] is a molecular marker method that has been shown to yield conservative estimates of PyC quantity in different environmental matrices and was able to quantify PyC over a broad range of the combustion continuum [8]. Moreover, the BPCA method yields additional information about PyC quality, such as its degree of aromaticity and aromatic condensation, which is related to the temperature of pyrolysis [14-15]. Since the method is based directly on molecular separation, it also allows the further analysis of isolated PyC molecular compounds to determine their isotopic composition, including ^{13}C and ^{14}C [16-17].

The BPCA method employs nitric acid to break down the PyC polymers into a suite of BPCA monomers, which are then purified and chromatographically analyzed. This last step is commonly done by gas chromatography (GC) [11-13]. The amount of detected BPCAs in a sample then serves as an estimate of its PyC content. Recently, it was shown that the procedure could be simplified for highly organic seawater or charcoal samples by analyzing the BPCAs on a high-performance liquid chromatography system ($HPLC_{organic}$)

[4,15]. Liquid chromatography does not require the time-consuming, external carbon-introducing and sometimes incomplete derivatization, which is necessary for the GC method (a technical overview is given in the supplementary material).

Although the $HPLC_{organic}$ method works well with highly organic samples, analyses of more complex environmental matrices proved difficult due to interference from organic and inorganic substances. Moreover, the $HPLC_{organic}$ method runs at pH 8 and uses tetrabutylammonium bromide, an organic modifier that prohibits the potential use of mass spectrometry, including isotope analyses. It is possible to use ion exchange chromatography [17] in order to circumvent this issue at high pH, but this approach unfortunately suffers from laborious sample preparation and tedious solvent and column maintenance.

Here, we present an improved HPLC method (HPLC) that is able to reproducibly separate and quantify BPCAs in complex environmental matrices with varying amounts and types of organic matter contents as well as in highly organic samples. Its low pH allows separation without an organic modifier and the use of the here described mobile phases is favorable for subsequent isotopic analysis of BPCAs. Environmental PyC reference materials were measured for comparing the HPLC method with the previous GC method. To test for plausibility, we analyzed a peatland core from a location that is known for its wide range of organic matter and charcoal contents.

2. Materials and Methods

2.1. Environmental PyC reference materials

A suite of PyC reference materials from a previous intercomparison exercise [8,18] was used to compare HPLC and GC quantification of BPCAs. In particular, Aerosol (NIST Standard SRM 1649b - Urban Dust), Marine Sediment (NIST Standard SRM 1941b), Vertisol (Clay Soil), Chernozem (Silty Soil), Dissolved Organic Matter (DOM), Wood Charcoal (pyrolyzed *Castanea Sativa*), Grass Charcoal (pyrolyzed *Oryza Sativa*) and *n*-hexane soot were analyzed.

In order to compare the HPLC method with the previous GC method, their respective intra-laboratory reproducibility was quantitatively assessed with the coefficient of variation (CV) [19] by measuring the environmental PyC materials in replicates (Table 1).

2.2. Peatland core

2.2.1. Bulk core analyses

A 2.5 m long core was taken at Bega Swamp [20-21] (NSW, Australia, 36 ° 32 ' 1.79 " S, 149 ° 29 ' 55.12 " E) and was split in 5 cm sections. The material within sections was homogenized and then taken for charcoal analyses (wet) or BPCA analyses (dried).

2.2.2. Charcoal Counting on the fraction >125 μm

A standardized macrocharcoal (> 125 μm) counting procedure [22-23] was carried out in each depth interval. Charcoal pieces were counted [number of pieces / wet volume aliquot] but PyC was reported as concentration [g BPCA-C / g dry material], and thus cannot be compared directly. In order to correct for the water mass loss due to drying, the charcoal counts were normalized by the factor f, as explained below. Furthermore, the logarithmic distribution of the charcoal counts was corrected. Both corrections helped to produce more comparable measures for the presence of charcoal, either reported as counts or as BPCA-based PyC concentrations (equation 1).

$$\log\left\{Charcoal\ counts\ \left[\frac{pieces}{aliquot}\right] * f\right\} \propto Pyrogenic\ carbon\ \left[\frac{BPCA-C\ [g]}{dry\ sample\ weight\ [g]}\right] \quad \text{(equation 1)}$$

where: $$f = \frac{1}{dry\ sample\ weight\ [g]}$$

2.3. BPCA analyses with the HPLC method

For the here presented HPLC method, we weighed dried and milled samples containing approximately > 1 mg TOC and digested the samples directly with nitric acid (65%, 8 h at 170 °C). The resulting solution, containing the BPCAs, was filtrated over ashless cellulose filters. The solution was further cleaned by a cation exchange resin and freeze dried to remove the acid. The freeze-dried residue was then redissolved in methanol/water (1:1) and eluted over a C18 solid phase extraction cartridge (Supelco, U.S.A.) to remove apolar compounds, after which it was dried again and transferred to the HPLC vials in ultrapure water.

Chromatographic BPCA separation was carried out with an Agilent 1290 Infinity HPLC system (Santa Clara, U.S.A.), equipped with an Agilent Poroshell 120 SB-C18 column (100 mm x 4.6 mm). Mobile Phase A consisted of orthophosphoric acid (Sigma-Aldrich, U.S.A) dissolved in water and buffered with NaH_2PO_4 (Sigma-Aldrich, U.S.A) to a pH-value of 1.2. Pure acetonitrile (Scharlau, Spain) was used as the mobile phase B (c.f. supplementary data for mixing gradients). Alternatively, a purely aqueous gradient to pH = 4.7 can be used if it is important to work without organic solvents, e.g. for subsequent on-line oxidation to perform carbon isotopic analyses on the BPCAs (supplementary data). Figure 1 depicts the retention times of the BPCA target components for three different samples. A photo diode array detector (DAD) was used for peak identification (absorbance spectra 190 – 400 nm), in concert with retention times of BPCA standards. The 240 nm and 216 nm wavelengths (slit width: 8 nm) were used to record the chromatograms for subsequent quantification.

2.3.1. HPLC method evaluation

We tested the HPLC method further in-depth with respect to (I) quantification of BPCAs, (II) required sample quantities and (III) recoveries of BPCAs after the simplified pretreatment steps prior to HPLC injection.

For the chromatographic quantification of the BPCAs (I), we compared the more reliable standard addition quantification approach with the less laborious external standard quantification approach [24]. The two approaches yielded essentially the same results in case of the matrix containing Chernozem reference sample (supplementary data), suggesting that the simpler external standard quantification is suitable for the HPLC method.

176 The linearity of the HPLC method (II) was evaluated by measuring two
177 reference matrix samples (Chernozem, Vertisol) with differing sample
178 amounts. Quantification was linear, even when working with less than 100 mg
179 of soil sample (supplementary data), corresponding to roughly 1 mg of
180 organic carbon per sample.

181 Recovery of the BPCAs (III) after pretreatment (cation exchange resin, solid
182 phase extraction, transfer and handling) was assessed by treating well-known
183 amounts of BPCA standard solutions (Sigma-Aldrich, U.S.A) the same way as
184 the samples. No systematic proportional error was observed (supplementary
185 data), i.e. the recovery is independent from the amount of sample or its
186 BPCA content. There is, however, a small systematic constant error (< 14 μg)
187 for all BPCAs, which is probably due to losses during handling.

188

189

190

3. Results and Discussion

3.1. HPLC-BPCA method for environmental samples (HPLC)

The HPLC sample pretreatment procedure and separation technique allowed baseline separation of all BPCA target components in all the analyzed environmental samples (e.g. Fig 1). Thus, it also represents an improvement compared to previous HPLC methods (e.g. $HPLC_{organic}$) because, to the best of our knowledge, no baseline separation of all BPCAs has been achieved with liquid chromatography before [15,17].

The HPLC method resulted in a more consistent quantification of PyC reference materials as compared to the well-established GC method. In a repeated experiment, the Chernozem reference sample was measured multiple times (n = 28) in our laboratory using both the GC [13] and the HPLC procedure. The HPLC method showed a much smaller coefficient of variation (CV = 6 %) compared to the GC method (CV = 22 %), translating into better reproducibility (Fig 2, right side). The improved intra-laboratory reproducibility of the HPLC method versus the GC method was further confirmed when we compared the replicated (n = 2 - 3) PyC-values of eight reference materials analyzed with both methods (Fig 2). The samples had been analyzed by two to three different laboratories using the GC method [8] with a respective mean intra-laboratory CV of 16 - 23 % (min: 6 %, max: 43 %; Fig 2, bottom). In contrast, the HPLC method showed a mean intra-laboratory CV of only 5 % (min: 1 %, max: 10 %) and thus a much better intra-laboratory measurement reproducibility.

The BPCA yields also showed a consistent pattern between the HPLC and the GC method: The HPLC method always detected an amount that was at least

217 equal to the maximum detected by the other laboratories using the GC
218 method. In the case of the two charcoal standards, the HPLC method
219 detected even slightly more than the maximum of the GC measurements. It
220 seems, therefore, that the HPLC method always captures the maximal yields
221 of BPCA, resulting in a lower variation of the replicates.

222 These results are in agreement with a previous GC-$HPLC_{organic}$ comparison
223 using almost purely organic (char) samples [15]. Higher reproducibility and
224 yields of the $HPLC_{organic}$ method were, at that time, attributed to fewer losses
225 during sample handling and possibly the omission of the trifluoracetic acid
226 and the derivatization step (cf. supplementary data). This probably also holds
227 true in the case of the environmental matrix samples measured with the
228 presented HPLC method.

229 The here presented HPLC method has additional advantages over the GC
230 method. While it requires less sample material per measurement, higher
231 sample throughput is achieved because of the simplified sample preparation
232 and reduced chromatographic analysis time. Additionally, entirely prepared
233 samples can now be stored in the vials for at least three months, which is
234 useful in case of intermittent instrument access, or repeat measurements
235 made later. In the GC protocol, samples had to be laboriously preprocessed
236 immediately before measurement (cf. supplementary data).

237

238 ### 3.2. PyC in the peatland core

239 We considered the Bega Swamp peatland core to be an ideal test sample for
240 the HPLC method because it spans a wide range of TOC contents (0.4 % – 42
241 % TOC), and because its wildfire history is well known [25]. Although TOC
242 contents varied widely, chromatographic separation of BPCAs was excellent
243 and PyC could be reliably quantified throughout the whole core.

The PyC quantification revealed very plausible site characteristics. Normalizing the PyC content to dry sample mass (Fig 3e) mirrored the overall trend of TOC because the PyC/TOC ratio stayed relatively constant. However, in contrast to the TOC, the PyC values deviated between the bulk sediment and the > 125 μm fraction for the layers above *ca.* 130 cm (Fig 3e, striped area). The grain sizes > 125 μm, were enriched in PyC in these upper layers. When we additionally consider the fact that the large grain size fraction (> 125 μm) dominates the upper part of the sediment (Fig 3b), it becomes evident that the majority of total PyC in the upper part of the peatland must have consisted of relatively large particles. The size distribution of fire residue particles in sediments is often used to reconstruct the distance of past fire events [26-28]. Thus, the larger pyrogenic particles present in the upper 130 cm indicate more local fires in the last *ca.* 4000 years [20], which appears very plausible because it coincides with the onset of drier conditions and the expansion of the *Eucalyptus/Casuarina* forest at this site [25].

Without venturing too far into the large field of wildfire reconstructions (e.g. Conedera et al. [29]), we aimed for an additional, simple plausibility check: Does the BPCA method detect similar quantities of fire residues to the charcoal count method for the same sample? The two measures capture two different aspects of charcoal (particle count vs. molecular mass concentration) and cannot be compared directly (section 2.2.2). Still, both values basically show a similar pattern for the peatland core (Fig 3e/f). Since BPCAs are a molecular marker for charcoal [11], correlation between the charcoal count data and the molecular marker can be expected, confirming the plausibility of the BPCA measurements obtained with the HPLC method presented above.

4. Conclusion and Outlook

The presented HPLC method for various kinds of environmental samples requires less sample material than the widely used GC method and is thus particularly suitable for small samples, e.g. from sediment cores or aerosol collectors. Despite the reduction of sample amounts and the simplification of sample pretreatment, the HPLC method still showed higher reproducibility and very plausible PyC values as compared to the commonly used GC method or when applied to samples from a peatland site.

The BPCA isolation and separation method applied here (HPLC) can be used to purify individual BPCA for subsequent radiocarbon analyses (unpublished results). Moreover, when the method is set up with a pH gradient as shown above, it is possible to measure the ^{13}C of the PyC-derived BPCAs by on-line isotope-ratio monitoring. Besides PyC quantity and quality, the PyC isotopic information may yield valuable supplementary information about the burned biomass fuel and its age. Thus, the field of possible applications for the HPLC method is large and includes paleo-environmental reconstructions using sediment cores, the investigation of archaeological artifacts, or biochar and soil carbon studies.

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Author Contributions

D.B.W. designed and conducted the study, analyzed the data and wrote the paper. M.D.H. and R.H.S. gave conceptual and technical support. S.G.H. provided the peatland core and charcoal counts and M.W.I.S. designed the study and gave conceptual advice.

Table 1

The environmental PyC reference materials that were used for the comparison of the HPLC method with the GC method. Chernozem and Vertisol were also used for the HPLC method evaluation (chromatographic quantification approach and linearity). The GC data from the different laboratories come from Hammes et al. [8,18] and the GC data from the repeated experiment were partially published in Schneider et al. [13].

PyC Reference Material	Number of Replicates			
	HPLC	GC		
	our lab	our lab	other lab 1	other lab 2
Aerosol	2	3	3	--
DOM	3	2	4	--
Marine Sediment	3	2	2	--
Vertisol	3	3	2	3
Chernozem	3	3	2	2
Grass Charcoal	2	3	2	2
Wood Charcoal	3	3	2	2
Soot	3	3	2	3
repeated experiment:				
Chernozem	28	28	--	--
HPLC method evaluation:				
Chernozem	22	--	--	--
Vertisol	9	--	--	--

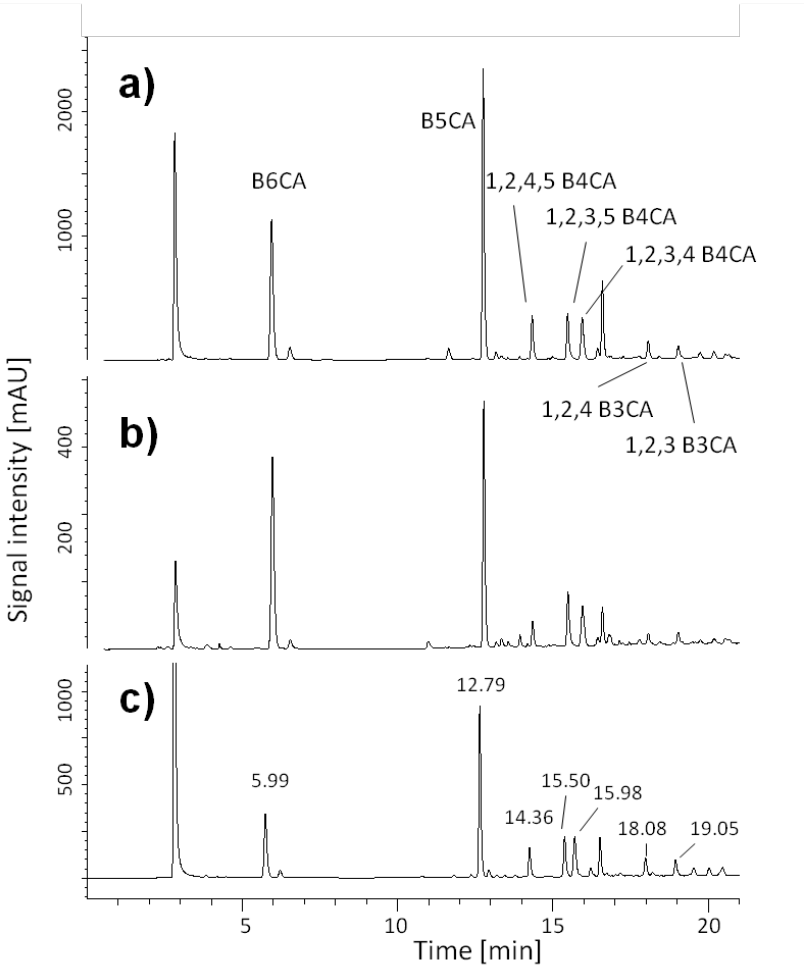

Figure 1

Chromatograms of nitric acid oxidation products according to the HPLC preparation and analysis method: a) Sediment from Bega Swamp in 80 – 85 cm depth, sieved to > 125 *µ*m; b) Chernozem; c) Grass charcoal (*Oryza Sativa*). Baseline separation was achieved for all the BPCA target components (B6CA; B5CA; 1,2,4,5-, 1,2,3,5-, 1,2,3,4-B4CA; 1,2,4-, 1,2,3-B3CA) in all the analyzed samples.

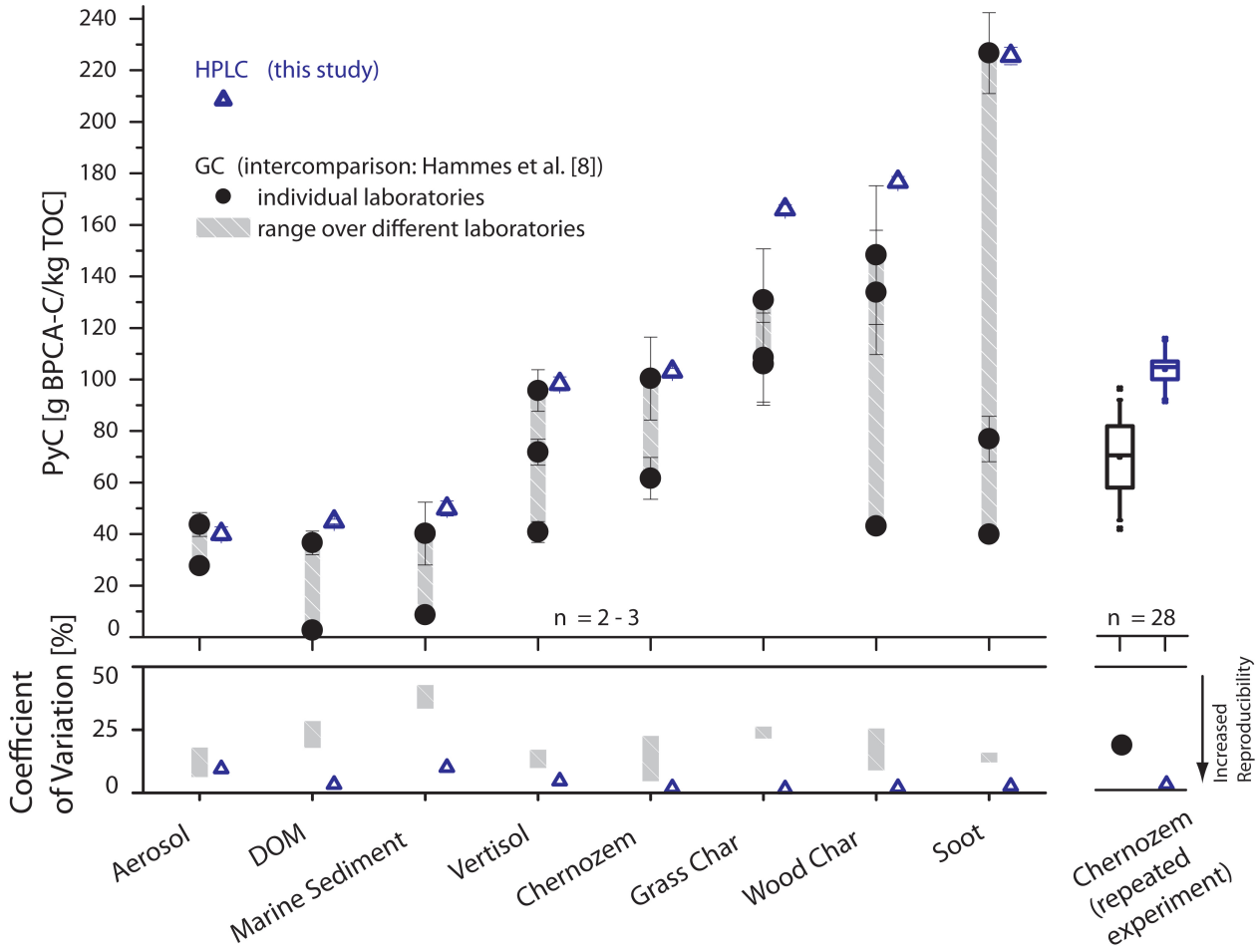

Figure 2

Replicated PyC measurements of different PyC reference materials with the HPLC and GC method. Error bars for the HPLC method are mostly smaller than symbol size. Triangles in this and the following figure represent PyC quantifications that were achieved with the HPLC method. Intra-laboratory measurement reproducibility was higher for the HPLC method than the GC method, as can be seen by the lower coefficient of variation. Moreover, the HPLC method always detected the maximum

amount of PyC (maximal BPCA yield) in the reference materials that was detected with the GC method in the different laboratories.

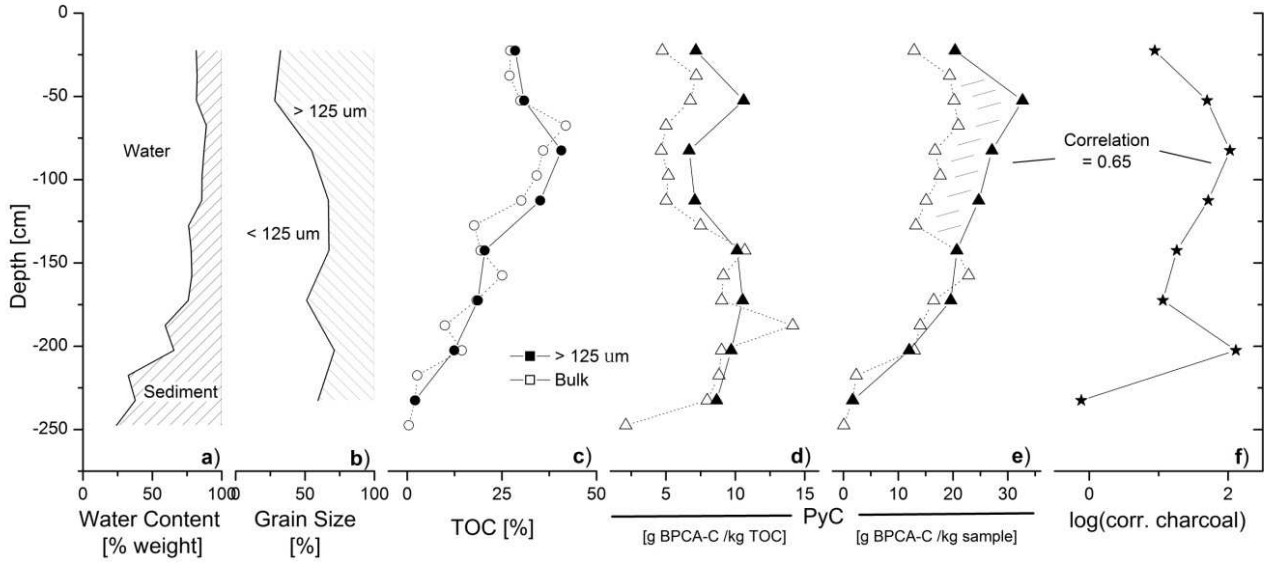

Figure 3

Bega Swamp core characteristics with respect to water content, grain size, TOC, PyC and charcoal counts. Empty symbols show the values for the bulk sediment while filled symbols represent the values for the fraction > 125 μm. Analytical errors for TOC (n = 2) and PyC (n = 3) are smaller than symbol size while charcoal counts were not replicated.

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Section 2.3

The commonly used GC method for environmental samples, the $HPLC_{organic}$ method for highly organic samples and the here presented HPLC method for various environmental samples. Note that Yarnes et al. [17] presented an ion exchange chromatography approach that is not listed here.

Nr	Work step/description	GC–BPCA	$HPLC_{organic}$–BPCA (only for highly organic samples)	HPLC–BPCA
		Glaser et al. (1998), Brodowski et al. (2005), Schneider et al. (2010)	Dittmar (2008), Schneider et al. (2011)	this study
1	Sample preparation before HNO_3 digestion	Trifluoracetic acid (TFA) digestion (4 h at 105 °C), filtration: collect sample on glass fiber filter (GF 6, Schleicher and Schuell, Dassel, Germany) and rinse wih excess of water, dry (2 h at 40 °C)		
2	HNO_3 digestion, conversion to BPCA	2 ml 65% HNO_3 (8 h at 170 °C in oven)	2 ml 65% HNO_3 (8 h at 170 °C in oven)	2 ml 65% HNO_3 (8 h at 170 °C in oven)
	Solid to acid ratio, mg C ml^{-1}	1–25	1–2.5	1–25
3	Sample preparation after HNO_3 digestion	Filtration over ashless cellulose filter (589/3, 110 mm diameter, Schleicher and Schuell, Dassel, Germany) into 10 ml volumetric flasks, fill up with deionized water	Drying at 60 °C under N_2 stream and dissolution in methanol/water (1:3), further dilution with mobile phase A	Filtration over ashless cellulose filter (589/3, 110 mm diameter, Schleicher and Schuell, Dassel, Germany) into 50 ml volumetric flasks, fill up with deionized water
		Addition of internal standard phtalic acid, cleaning with cation exchange resin (Dowex 50W X 8, 200–400 mesh, Fluka, Steinheim, Germany), freeze drying for acid removal, transfer to GC vials with four times 1 ml metha		Cleaning with cation exchange resin (Dowex 50 W X 8, 200–400 mesh, Fluka, Steinheim, Germany), freeze drying for acid removal.
				Redissolution in methanol/water (1:1), cleaning through solid phase extraction tubes (DSC–18, Supelco, USA), drying and transfer to LC vial in ultrapure water
4	Derivatization	100 µl BSTFA + TMCS, 10 µl pyridine (2 h at 80 °C + storage for 24 h)		
5	Chromatographic analysis			
	Mobile phase A	He	Ortho phosphoric acid (50%) 1 ml^{-1} Tetrabutylammonium bromide (TBAB) 2 g^{-1} – Dissolved in water – Adjusted to pH 8 by slowly adding 1 M NaOH	Ortho phosphoric acid (85%) 25 ml^{-1} – Dissolved in water – Buffered with NaH_2PO_4 (ca. 250 $mg\,l^{-1}$) – target value pH 1.2
	Mobile phase B	–	Mobile phase A + 75% MeOH	Acetonitrile
	Injection volume	1 µl	20 µl	1 µl
	Injections per sample replicate	2	1	1
	Flow rate	0.8 ml min^{-1}	0.18 ml min^{-1}	0.4 ml min^{-1}
	Column temperature	100–300 °C	16 °C	15 °C
	Column/quantification	Agilent DB–5 (50 m, diameter 0.2 mm)/flame ionization detector (FID)	Waters Atlantis T3 3 µm (150 mm, diameter 2.1 mm)/ UV absorption at 240 nm	Agilent Poroshell 120 SB–C18 (100 mm, diameter 4.6 mm)/ UV absorption at 240 nm
	Identification	Retention time, GC–MS	Retention time, absorbance spectra 220–380 nm	Retention time, absorbance spectra 190–400 nm
	Quantification	External standards of BPCAs with correction for losses by Internal Standard	External standards of BPCAs	External standards of BPCAs
A	Storage for remeasurements	after filtration (Step 3), acid extract diluted with water, <1 month	not tested	after transfer to LC vial (Step 3), dissolved in ultrapure water, ready for LC injection, >3 months
B	Approximate preparation time per sample batch	4–5 days	2–3 days	2–3 days

Section 2.3.

Mobile Phase mixing gradients (A: orthophosphoric acid buffered with NaH_2PO_4 to a pH-value of 1.2; B: pure acetonitrile) used for the HPLC method for various environmental sample materials.

Time [min]	Mobile phase B [vol %]
0	0.5
5	0.5
25.9	30
26	95
28	95
28.1	0.5
30	0.5

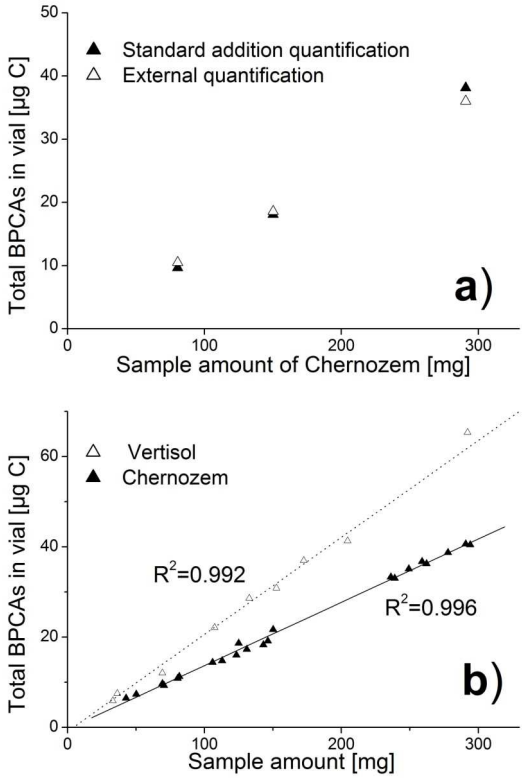
If the aim is to minimize the introduction of external carbon (e.g. for subsequent ^{13}C or ^{14}C measurements of the separated BPCAs), purely aequeous mobile phases with a pH gradient can be used:

A: 40 ml H_3PO_4 (85%) l^{-1} (target pH: 1.12)

B: 1560 mg NaH_2PO_4 l^{-1} (target pH: 4.7)

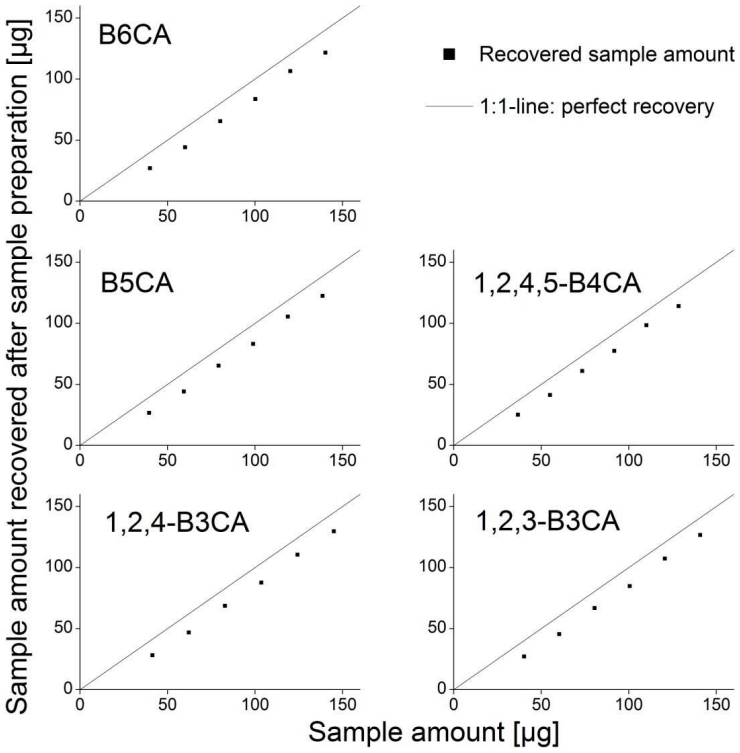
Time [min]	Mobile phase B [vol %]
0	1
5	1
10	35
18	100
31	100
31.1	1
35	1

Section 2.3.1.



The less laborious external standard quantification yields the same BPCA quantity as the standard addition quantification in a Chernozem for three different sample amounts (a). Linearity of the HPLC method with differing sample amounts for two soil samples: In these two cases, less than 100 mg soil sample (less than 1 mg TOC) is required for a reliable BPCA quantification in the linear measurement range (b).

Section 2.3.1.



Quantitative recovery of BPCA standards after the sample preparation steps. Losses are small and constant over different sample amounts and very similar for the different BPCA.